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TOPIC HIGHLIGHT

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Immunopathogenesis of inflammatory bowel disease

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Abstract

Crohn's disease and ulcerative colitis are chronic relapsing immune mediated disorders that results from an aberrant response to gut luminal antigen in genetically susceptible host. The adaptive immune response that is then triggered was widely considered to be a T-helper-1 mediated condition in Crohn's disease and T-helper-2 mediated condition in ulcerative colitis. Recent studies in animal models, genome wide association, and basic science has provided important insights in in the immunopathogenesis of inflammatory bowel disease, one of which was the characterization of the interleukin-23/Th-17 axis.

Key words: Crohn's disease; Ulcerative colitis; Innate and adaptive immune system

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INTRODUCTION

Inflammatory bowel disease (IBD) results from a complex series of interactions between susceptibility genes, the environment, and the immune system. The clinical features of the disease, histopathological findings, and the therapeutic efficacy of immunosuppressive drugs indicate an involvement of the immune system in the pathogenesis of the disease. Various components of the mucosal immune system are implicated in the pathogenesis of IBD. These components include luminal antigens, intestinal epithelial cells (IECs), cells of the innate and adaptive immune system, and their secreted mediators. An overview of the working hypothesis of IBD is depicted in Figure 1. Either a mucosal susceptibility or defect in sampling of gut luminal antigen leads to activation of innate immune response, possibly mediated by enhanced Toll-like receptor (TLR) activity. The stimulated dendritic cells (DC) then mediate the differentiation of naïve T-cells into effector T-cells. Crohn's disease (CD) is a predominately Th1 and Th17 mediated process, while ulcerative colitis (UC) appears to be predominately mediated through Th2 and NK T-cells. In this review, the effects of these components in the immunopathogenesis of IBD will be discussed.

BACTERIA AND THEIR ASSOCIATED MOLECULES IN THE INTESTINE

The distal ileum and colon contain high concentrations of bacteria (> 10^{12} organisms/g). These may include pathogens that could be directly responsible for initiating and promoting IBD in the context of an underlying genetic mucosal or immune defect. Studies have shown that there are differences in the microbiota between healthy and IBD subjects (Table 1). One of the differences is that there is a decrease biodiversity in IBD compared to healthy subjects by 30%-50%[1]. The reduction in diversity in inflammatory bowel disease was due to loss of normal anaerobic bacteria such as *Bacteroides*, *Eubacterium*, and *Lactobacillus* species[1]. Diversity is generally thought to be desirable by conferring resiliency to an ecosystem[2]. For example diversity would provide functional redundancy in the microbial community to ensure that key processes such as breaking down nutrients and preventing random chaotic fluctuation of bacterial subpopulations. Restriction of biodiversity in the human gut may lead to dysbiosis and result in mucosal insult. A second difference is that there are fewer *Firmicutes* in IBD compared to healthy subjects; 13 distinct *Firmicutes* ribotypes were identified in CD microbiota compared to 43 in healthy microbiota ($P < 0.025$)[3]. *Firmicutes* are Gram-positive class of bacteria that include the genus *Clostridium* and *Bacillus*. Third, there are pathogens that are found in increasing frequency in IBD and have been implicated to associate with its development. These pathogens include *Pectinatus*, *Sutterella*, *Fusobacterium*, *Verrucomicrobium*, various *Clostrodia*, *Mycobacterium paratuberculosis*, *M. paramyxovirus*, Listeria monocytogenes, and Helicobacter hapaticus[4,5]. Despite the differences in the microbiota, one must keep in mind that the dysbiosis seen in IBD patients may not be causal, but simply reflect the different ecological conditions of the inflamed gut such as changes in pH,

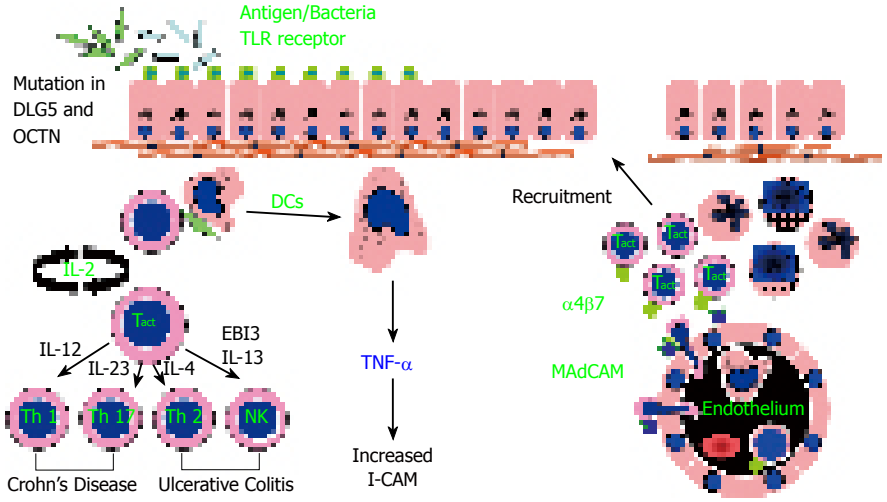

Figure 1 Working hypothesis of inflammatory bowel disease. A defect occurs in sampling of gut luminal antigen, possibly mediated by enhanced Toll-like receptor activity and controlled by other genetic factors (mutations in DLG5 and OCTN). Over-response to antigens results in stimulated dendritic cells (DC) that recruits and generates various T-cell subtypes, which then initiate a cascade of immunologic events leading to mucosal inflammation. Adhesion molecules such as intercellular cell adhesion molecule 1 (ICAM1) are important for circulating mononuclear and polymorphonuclear cells to adhere and migrate to the inflamed gut mucosa. Crohn's disease (CD) is a predominately Th1 and Th17 mediated process, while ulcerative colitis (UC) appears to be predominately mediated through Th2 and NK T-cells.

Table 1 Comparison of microbiota between healthy and IBD subjects

Healthy subjects	IBD subjects
High biodiversity	Low biodiversity
Stable microbiota	Dysbiosis
Increased gut commensals	Increased gut pathogens
Higher firmicutes	Lower firmicutes

redox potential, substrate availability, *etc.*

The importance of the microflora in the induction of and maintenance of disease has been demonstrated in murine model of colitis. For example, mice that are deficient in the cytokines IL-2 or IL-10 or rats containing the *HLA-B27* transgene develop IBD in the presence of a normal microflora but not in a sterile germ-free environment[6-8]. Moreover, experimental colitis is attenuated when animals are treated with broad spectrum antibiotics[9]. Different bacteria may lead to different types of colitis in the same genetic host. In *IL-10*$^{-/-}$ mice, *E. coli* induced proximal colitis whereas *Enterococcus faecalis* lead to distal colitis[10]. Indirect evidence suggesting a role of the intestinal microbiota in IBD came from a double blinded, randomized controlled trials that showed imidazole antibiotics decrease the risk of post-operative recurrence of CD[11].

The surfaces of microorganisms typically bear repeating pattern of molecular structures as do their nucleotides. These motifs are designated as Pathogen Associated Molecular Patterns (PAMP). Some of the PAMPs include complex macromolecules such as lipopolysaccharide (LPS), peptidoglycan (PGN), polypeptides (flagellin), and nucleic acid (CpG rich DNA). Receptors of the PAMPs are called pattern recognition receptors (PRR). Toll like receptors (TLR) are examples of PRR. TLR recognition usually triggers the innate immune system, leading to an inflammatory response. Interaction between PAMPs and PRR are illustrated by the CpG dinucleotides activation of innate immunity *via* TLR9 (Figure 2). Oligonucleotides containing CpG motifs (CpG-ODN) prevented lesions and reduced the release of inflammatory cytokines when given before the DSS-induced colitis[12-14]. However, if CpG-ODN is given after DSS-colitis induction, the colitis is worsened[13-15]. In TLR9-deficient mice, the onset of DSS-induced colitis was not prevented, but chronic inflammation was reduced[12,15]. This is due to the dual anti-inflammatory effect of TLR9 signaling that is mediated through type I interferon (IFN)[16].

The study of autoantibody has provided evidence that immunologic responses to bacterial products is involved in the induction of inflammatory bowel disease. Reactivity to bacterial antigens was initially shown by findings of modest increase in serum antibodies to 7 bacterial pathogens in a group of CD patients[17]. Subsequently, several studies showed serum responses to various bacterial antigens and loss of tolerance to pathogenic as well as commensal bacteria in clones derived from peripheral and lamina propria T-cells from CD subjects[18-22]. This indicates that disordered features of T-cell microbial recognition and effector function are likely to be important to IBD disease biology.

Antibodies to specific bacterial antigens are clustered into 4 major groups in IBD patients: (1) antibody responses against oligomannan [anti-Saccharomyces cerevisiae (ASCA)][23]; (2) antibody responses to both *Escherichia coli* outer membrane protein C (anti-OmpC) and a CD-related protein from *Pseudomonas fluorescens* (anti-CD-related bacterial sequence)[24,25]; (3) antibody response to nuclear antigens [perinuclear antinutrophil cytoplasmic antibody (pANCA)][26]; or (4) antibody response to the flagellin, CBir1[27]. These antibodies provides immunophenotypic associations to distinguish between UC and CD. CD patients with high levels of IgG and IgA ASCA and the absence of pANCA have more aggressive, small bowel fistulizing and fibrosing disease, and patients with high-level pANCA, in the absence of ASCA, have an ulcerative colitis-like colonic disease[28-30]. In a prospective study, pANCA expression is associated with development of pouchitis after ileal pouch-anal anastomosis (IPAA)[30]. The site of pANCA production has been localized to the gastrointestinal mucosa and was not found in detectable amounts in the periphery, suggesting the importance of mucosal immune response to luminal antigen[31]. In fact, absorption of either human or mouse pANCA-positive sera with enteric bacterial antigens greatly reduced or abolished the specific perinuclear staining of pANCA,

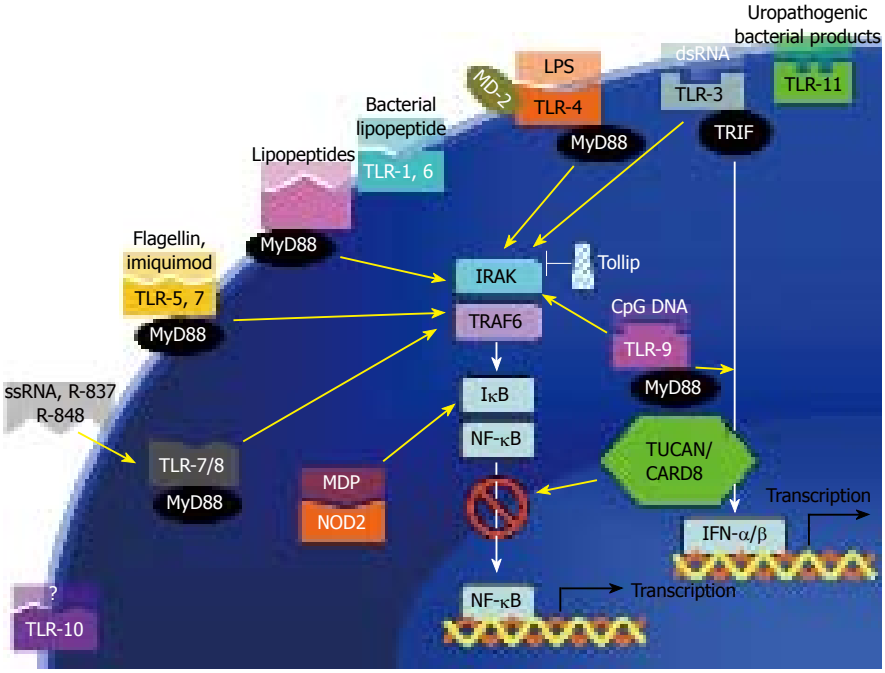

Figure 2 PRRs and their corresponding ligands. Toll-like receptors on the cell membrane (TLR-1, -6, -10, and -11) and intracellular (TLR-7, -8, and -9) selectively bind to various bacterial, viral, or fungal components. A major convergent pathway is through myeloid differentiation primary response protein MyD88, which activates NF-κB. The death domain of MyD88 then recruits downstream IL-1 receptor-associated kinase (IRAK) to the receptor complex. IRAK is then autophosphorylated and in turn recruits TNF receptor-associated factor 6 (TRAF6). TRAF6 then activates kinases including NF-κB-inducing kinase (NIK) and mitogen-activated protein kinase/ERK kinase kinase 1 (MEKK1). Inhibitor of NF-κB degradation (IκB) is subsequently phosphorylated and degraded, resulting in NF-κB nuclear translocation. NF-κB then activate genes involved in inflammatory response including IL-1b, TNF, IL-6, IL-8, and ICAM1. Toll-inhibitory protein (Tollip) is one of the negative regulators of the innate immunity. Activation of type I IFN (IFN-α/β) also has anti-inflammatory function in colitis.

indicating that pANCA seroreactivity reflects a cross-reactivity to enteric bacterial antigens[32].

The flagellin, CBir1, has been identified as an immunodominant antigen that is capable of inducing severe colitis in mice and eliciting antibody responses in approximately 50% of CD patients[33]. In contrast, patients with UC or other inflammatory gastrointestinal diseases and control subjects have little or no reactivity to CBir1[33]. Serum responses to CBir1 independently identify a subset of patients with small-bowel, internal-penetrating, and fibrostenosing CD that have no correlation to levels of ASCA, OmpC, and pANCA[23]. Molecular studies in CBir have also shed light on the underlying immunopathophysiologic mechanisms related to this antigenic response. CBir involves both the innate and the adaptive immune system. CBir flagellin is found to be the ligand for TLR5 (Figure 2)[34]. Bacterial flagellin is a structural protein that makes up flagella, which functions in bacterial chemotaxis and adhesion of host tissues. TLR5 is expressed on the basolateral surface of intestinal epithelial cells and hypothesized to be important in the recognition of invasive flagellated bacteria at the mucosal surface. TLR5-flagellin interaction leads to the activation of the transcription factor NF-κB, resulting in the transcriptional induction of proinflammatory cytokines and maturation of human dendritic cells (Figure 1)[35,36]. The expression of antibodies to CBir1 and transferring of a CBir1-specific CD4 + Th1 cells to induce colitis in mice is indicative of an adaptive immune response to this antigen[27].

These results show that some endogenous micro-organisms provide antigenic stimulation through PAMP activation of PRR to maintain intestinal homeostasis: either inflammation through the release of inflammatory cytokines or mucosal protection through type I IFN. In addition, serum reactivity to bacteria and their associated molecules may indicate a predisposition to certain mucosal antigen that could reflect the clinical manifestation and type of IBD. These distinct antibody response patterns to either UC or CD indicate unique pathophysiologic mechanisms to IBD.

INTESTINAL EPITHELIAL CELLS

The 400-mm^2 single layer of gut epithelial cells is the primary cellular barrier to prevent antigens from encountering the immune system. The gut maintains an extensive and active gut-associated lymphoid tissue (GALT). Peyer's patches (PP) are aggregates of lymphoid tissue that are interspersed at intervals just beneath the gut epithelium. They are comprised of B-cell follicles located under specialized areas, or 'domes,' of epithelium known as follicular-associated epithelium (FAE), with T-cell zones occupy the areas between follicles[37]. FAE contains cells called multi-fenestrated or M cells whose function is to transport luminal antigen into the dome area of the follicle[37]. Dendritic cells (DCs) are antigen presenting cells that act as sentinels, sending processes between gut epithelial cells and sample commensal and pathogenic bacteria[38]. Defects in the epithelial barrier may allow GALT to be exposed to excess or harmful luminal antigens, resulting in the production of pro-inflammatory cytokines such as TNF-α and mucosal inflammation.

Tight junction between epithelial cells allows for the selective entry of fluids, nutrients, and micro-organisms. Normal gut permeability is dependent on an intact epithelium, surface mucus, peristalsis, and the secretion of host protective factors. Alterations in gut permeability factors have been reported. Two genes that are associated with Crohn's disease which affect mucosal permeability are the IBD 5 gene organic cation transporter (*OCTN*) and guanylate kinase *DLG5* (Figure 1). Missense mutations in the *OCTN* gene form a haplotype that is associated with susceptibility to Crohn's disease and the mutant protein has impaired ability to pump xenobotics and amino acids across cell membrane[39]. The other protein, *DLG5*, is a scaffold protein involved in the maintenance of

epithelial integrity. Two distinct haplotypes in the *DLG5* gene represented by nonsynonymous single-nucleotide polymorphism in the gene are associated with IBD[40]. It is proposed that the mutation probably impedes scaffolding of *DLG5* and thus, affect epithelial polarity[40]. Altered mucus production has also been found in IBD patients with thinner than normal colonic mucus layer in UC and a thicker than normal layer in CD[41]. Several studies have shown that the there is an increased number of adherent bacteria in IBD patients[42-44]. This relationship holds true in both the mucus layer and at the epithelial surface where bacteria are less abundant. It is possible that the disruption of the function of *OCTN* and *DLG5* and abnormal mucus composition alters the gut permeability. Altered gut permeability may lead to increased bacteria adherence and inappropriate exposure of the mucosal immune system to bacterial products causing inflammation.

INNATE IMMUNITY

Studies described above indicate that microbial structures can cause gut mucosal inflammation by involving the innate immune system. Adhesion molecules such as intercellular cell adhesion molecule 1 (ICAM1) are important for circulating mononuclear and polymorphonuclear cells to adhere and migrate to the inflamed gut mucosa (Figure 1)[45]. Proinflammatory molecules are preferentially produced by innate immune cells that have migrated to the inflammatory mucosa owing to increased expression of PRR such as TLR[46]. Antigen-presenting cells such as dendritic cells (DCs) and macrophages possess TLRs with different specificities for microbial products and induce innate immune response. Immunopathogenic role of TLR signaling in IBD is in the process of being elucidated.

NUCLEOTIDE-BINDING OLIGOMERIZATION DOMAIN (NOD) PROTEINS

NOD proteins are a distinct subset of PRRs that have important roles in innate immunity as cytoplasmic sensors of microbial components, allowing for regulation of inflammatory processes and apoptosis[47]. The importance of these PRRs is highlighted by the fact that mutations in the gene that encodes NOD2 occur in a subset of patients with CD and polymorphisms in the gene that encodes NOD1 are associated with IBD[48-50]. The NOD1 protein (encoded by caspase-recruitment domain 4 gene, *CARD4*) and NOD2 protein (encoded by CARD15) are expressed by antigen-presenting cells (APCs) and epithelial cells[47]. NOD2 is a general sensor of most bacteria because it recognizes muramyl dipeptide (MDP), which is a component of both Gram-positive and Gram-negative bacteria[51]. In contrast, NOD1 senses mostly Gram-negative bacteria because the ligand for NOD1, peptidoglycan (PGN) derived peptide γ-D-glutamyl-mesodiamino-pimelic acid (iE-DAP), is not present in Gram-positive bacteria except for *Listeria* and *Bacillus spp*[52]. The main outcomes of NOD1 and NOD2 activation is the activation of NF-κB and mitogen-activated protein kinase (MAPK) pathway (Figure 2)[51,53-55]. In addition, activation of NOD1 by its ligands leads to the activation of JUN N-terminal kinase (JNK)[47]. Activations of NF-κB, MAPK, and JNK pathways lead to the production of pro-inflammatory mediators[47].

There are 3 models of how *CARD15* mutations are associated with CD, however, studies have shown inconsistencies in these models. The first view is based on the study that showed NOD2 is a negative regulator of IL-12 mediated by PGN[53]. Loss-of-function mutation in CARD15 results in an excessive NF-κB dependent IL-12 response by splenic macrophages to create a milieu that supports Th1 cell mediated colitis. This is supported by findings that production of IL-12p70 by peripheral blood monocytes derived dendritic cells in response to PGN is higher in patients with CARD15 mutation than in normal subjects[56]. In contrast to the study by Watanabe *et al*, another study that used bone-marrow derived macrophages did not result in enhanced proinflammatory cytokine production[55]. Other studies show that CARD15 mutation results in reduced anti-inflammatory cytokine responses (IL-10 and TGF-β) to several TLR ligands[57,58]. These indicate that NOD2 signaling should augment an anti-inflammatory response and not inhibition of TLR responses in normal individuals. However, defective anti-inflammatory response secondary to CARD15 mutation does not sufficient explain increased cytokine production that is characteristic of CD.

The second model suggested that a loss-of-function NOD2 lead to susceptibility to CD as a consequence of impaired host defense. This may be due to impaired NOD2-dependent α-defensin production by Paneth cells, and thus, lead to impaired control of pathogenic bacteria and inflammatory condition[59]. This is also supported by the finding that CD patients with CARD15 mutation have a more pronounced defective production of a-defensin-5 and -6[60]. Consistent with the impaired mucosal host defense, NOD2 deficient mice developed worse liver infection to *L. monocytogenes* when the pathogen was given orally compared with systemic administration[55]. However, there are several studies that did not support the idea that CD is due to impaired host defense as a consequence of NOD2 mutation. One of the strongest argument is the data that mice in with ablation Paneth-cell population or mice that lacks α-defensins develop evidence of acute or chronic inflammation[61,62]. In addition, NOD2-deficient mice that were administered *L. monocytogenes* did not cause mucosal infection[55].

The third model proposes gain-of-function mutations in the *CARD15* gene that are associated with CD. This possibility is supported by studies in mice carrying a mutation that is analogous to the most common CD susceptibility allele, 3020insC[63]. The mutant mice show increased NF-κB activation and enhanced production of IL-1β upon MDP stimulation of bone marrow-derived macrophages[63]. Inconsistent with the above study, peripheral-blood mononuclear cells isolated from CD patients that carrie the 3020insC allele show a defect in IL-1β secretion[64]. Additionally, epithelial cells transfected with 3020insC CARD15 mutation have defective NF-κB activation upon MDP stimulation[51,65].

There are several views and contradictory findings on the role of NOD protein in the pathogenesis of IBD.

Among the unanswered questions are whether CARD15 mutations confer a gain- or loss-of protein function. Perhaps the mutations have different effects in different context, i.e. in the APC *versus* epithelial cells or in the mucosa *versus* the periphery. More studies are needed to elucidate the immunopathogenesis of CARD15 mutations in IBD.

TOLL LIKE RECEPTOR AND ACTIVATION OF INNATE IMMUNE RESPONSE

Eleven TLRs have been identified which are characterized by three common structural features: (1) divergent ligand binding extracellular domain with leucine rich repeats; (2) short transmembrane region; (3) a highly homologous cytoplasmic Toll/interleukin 1 receptor (TIR) domain. Even though each type of TLR engages a different PAMPs, a major convergent pathway is through myeloid differentiation primary response protein MyD88, which activates NF-κB. Upon activation, TLRs form homodimers leading to a conformation change in the cytoplasmic TIR domain and recruitment of MyD88[66]. The death domain of MyD88 then recruits downstream IL-1 receptor-associated kinase (IRAK) to the receptor complex[66]. IRAK is then autophosphorylated and in turn recruits TNF receptor-associated factor 6 (TRAF6). TRAF6 then activates kinases including NF-κB-inducing kinase (NIK) and mitogen-activated protein kinase/ERK kinase kinase 1 (MEKK1)[67]. Inhibitor of NF-κB degradation (IκB) is subsequently phosphorylated and degraded, resulting in nuclear translocation of NF-κB[66]. NF-κB belongs to Rel family of DNA binding transcription factor that bind characteristic sequence motifs and activate genes involved in immune and inflammatory response[66]. This is illustrated in Figure 2.

Activation of NFκB stimulates expression of numerous molecules relevant to the pathogenesis of IBD including factors involved in the inflammatory response (IL-1b, TNF, IL-6, IL-8, ICAM1, and other chemokines and adhesion molecules), co-stimulatory molecules (CD40, CD80, CD86, and the inducible T–cell co-stimulator ICOS)[68]. In addition, inhibition of NF-κB can attenuate experimental colitis, illustrating its proinflammatory effect[69]. Other studies have shown that NFκB maintains gut epithelial homeostasis by exerting mucosa protective effects. For example, failure to activate epithelial cell NF-κB *in vivo* results in a significant increase in radiation-induced epithelial cell apoptosis[70]. Indirectly blocking NFκB activation *via* targeted deletion in *MyD88* gene worsens DSS induced colitis in mice in gut epithelial cells, this will be discussed in further detail below[71].

NEGATIVE REGULATORS OF INNATE IMMUNE RESPONSE

TLR signaling in the gut is kept under control by inhibitors of TLR signaling. One such negative modulator is Toll-inhibitory protein (Tollip). Prolonged exposure to LPS (most commensal organisms contain LPS in their cell wall) leads to elevated Tollip expression which makes intestinal epithelial cells poorly responsive to TLR-dependent response to commensal microflora[72]. Other negative regulators of TLR signaling include SIGIRR (single immunoglobulin-IR-related molecule or Tir8) and peroxisome proliferator-activated receptor-γ (PPAR-γ)[73,74]. Consistent with the biological plausibility that reduced expression of these inhibitors lead to IBD, intestinal inflammation is enhanced in SIGIRR-deficient mice[73]. In addition, expression of PPAR-γ is decreased in patients with active UC (but not CD) and up-regulated by 5-aminosalicylic acid[75,76].

Negative regulation of the host innate immune responses to the indigenous microflora maintains gut homeostasis. Key players in the negative mucosal regulation include interleukin-10 (IL-10) and IL-2, as evidenced by mice with deficiencies in these factors develop spontaneous intestinal inflammation, but are protected from intestinal disease when raised in germ-free environments[77-79]. This indicates that at steady state, pathologic consequences of immune activation by commensal microflora are constitutively inhibited by IL-10 and -2 dependent mechanisms. Colitis can be prevented when *IL-10*$^{-/-}$ mice are crossed to MyoD88 deficient mice, demonstrating that IL-10 maintains intestinal immune homeostasis by negatively regulating MyD88-dependent, commensal-induced inflammation[71]. In contrast, colitis in IL-2 deficient mice is independent of MyD88 and TRIF pathway. This indicates that the commensal dependent colitis in IL-2 deficient mice is driven by either nonclassical TLR-dependent signaling (independent of MyD88 and TRIF) or through a non-TLR innate pathway.

The development of colitis in IL-10 and IL-2 deficient mice is mediated by a pathologic T-helper type 1 (Th1) immune response through instructive signals induced upon innate recognition of microbes[79,80]. The Th1 response in IL-10 deficient mice is dependent on MyD88-dependent IL-12 or -23 p40 signaling[71,81]. In contrast, the aberrant Th1 response in the absence of IL-2 does not go through a classical microbial induction of MYD88/TRIF pathway and is also independent of IL-12 or -23 p40 signaling[71]. IL-27, a new bioactive member of the IL-12 cytokine family composed of an IL-12 p40 related polypeptide (EBI3) and a unique p28 subunit, may be the candidate instructive cytokine to drive a MyD88 independent Th1 response[71,82]. Its expression is associated with commensal-dependent, Th1 polarized colitis in IL-2 deficient mice[71]. Interestingly, IL-27 expression also is correlated with *IL-10*$^{-/-}$ Th1 polarized colitis, suggesting that IL-27 is regulated by both the TLR/MyD88 dependent and independent pathway[71].

The differential expression of TLRs may also in part explain how host intestinal mucosa discriminates between commensal (most of which are gram-negative bacteria that contain LPS in their cell wall) and pathogenic bacteria. Oral tolerance is believed to be controlled by antigen-presenting cells in the Peyer's patches by stimulating the activity of regulatory T-cells which suppresses adaptive immune response[83]. These Peyer's patch DCs produce IL-10 in response to inflammatory stimulation such as LPS, which is a ligand for TLR4. Unlike other TLRs, TLR5 is expressed mainly on CD11C+LPMC and not on conventional DCs or macrophages[84]. Interestingly, TLR4

expression is low in CD11C+LPMC[84]. Low expression of TLR4 in CD11C+LPMCs may prevent inappropriate immune response to commensal bacteria whereas TLR4 expression in Peyer's patch DCs leads to IL10 production and immune tolerance to commensal bacteria. In contrast, expression of TLR5 on CD11C+LPMC may allow mucosal inflammation following exposure to pathogenic flagellated bacteria.

ADAPTIVE IMMUNITY

It is established that the recognition of commensal-derived antigens by the adaptive immune system or its stimulation by the innate immune system play a key role in the pathogenesis of IBD. In particular, studies in genetically engineered systems and in inducible and adoptive transfer models of chronic intestinal inflammation have shown a key role of effector T lymphocytes for the inflammatory process in the gut. Importantly, both T helper 1 (Th1) and Th2 T cells have been shown to cause chronic gut inflammation, with CD having a predominately Th1 cytokine profile and UC having a Th2 cytokine profile (Table 2).

Differentiation of naïve T-cells into various T-cell subsets is summarized in Figure 3. IL-12, composed of P40 and p35 subunits, induces the formation of IFN-γ producing Th1 cells. In contrast, IL-4 induces STAT6 activation, promoting the expression of GATA-3, which feed forward to induce IL-4 expression and Th2 cell differentiation. IL-23 (composed of p19 and p40) promotes the development of an IL-17 producing CD4+ helper T cell subset through mechanisms that are distinct from the Th1 (STAT1, STAT4, and T-bet) and Th2 (STAT6) pathways[85,86]. Tregs, an immune-modulating subset of CD4+ T-cells, can suppress the differentiation and function of Th1 and Th2 cells. Interestingly, in the presence of IL-6, Treg-derived TGF-b can induce the differentiation of Th17 cells[87]. These T-cell subsets and their effect in IBD are discussed below.

T-CELL RESPONSES IN CROHN'S DISEASE

Immunologically it is proposed that inflammation in CD is the product of an exaggerated Th1 response mounted by genetically susceptible host in reaction to components of commensal bacteria[88]. The Th1 cytokine profile, including IFN-α, IL-12 (composed of p35 and p40), and TNF-α, is elevated in patients with CD[89-91]. Polarization of naïve T-cells towards Th1 type differentiation occurs through the activation of signal transducer and activator of transcription-1 (STAT1) and its stimulation of transcription factor T-bet, a Th1 "master switch" that upregulates and stabilizes the expression of IL-12[92-94]. IL-12 can amplify Th1 response by upregulating IL-18 on T-cells. IL-18 then stimulates NF-κB and AP1, and in synergy with STAT4 (activated by IL-12), transactivates IFN-α expression. The proposed pathway of TH1 polarization is summarized in Figure 3.

TNF-α and its family member are important in the pathogenesis of CD. Increased expression of TNF-α is seen in the intestinal mucosa of patients with Crohn's disease and Antibodies to TNF-α such as Infliximab decrease Th1 response in parallel with clinical and endoscopic healing[90]. The expression of LIGHT, another member of the TNF family, is also up-regulated in intestinal biopsies from CD patients and stimulation of the LIGHT receptor (herpesvirus entry mediator) induced IFN-γ production in lamina propria T cells, while blocking LIGHT inhibited CD2-dependent induction of IFN-γ synthesis, indicating a role for LIGHT in the regulation of IFN-γ and may be a way in which T-T cell interactions propagate intestinal inflammation[95,96].

TL1A is a recently identified member of the TNF superfamily that interacts with the death domain-containing receptor DR3 and found to be a strong co-stimulator of T-cells. Activation of T-cells by IL-12/IL-18 induces expression of DR3[97]. In T-cells, ligation of DR3 by TL1A activates NF-κB and leads to powerful co-stimulation of IFN-γ[98]. Co-activation is most evident for CD4+/CCR9+ T-cells which are enriched in the lamina propria and intraepithelial lymphoid compartment of the small intestine[99,100]. These T-cells are also enriched in the peripheral circulation of patients with CD and Celiac disease. IL-12 and IL-18 can also augment IFN-γ production in CD4+/CCR9+ T-cells[101]. In addition, peripheral CD4+/CCR9+ T-cells have been shown to express surface membrane (sm) TL1A, which appears to co-stimulate IFN-γ production independent of, but in synergy with IL12 and IL 18 (Figure 3)[102]. A recent study has provided a genetic perspective on the role of TL1A in

Table 2 Cytokine profile in IBD

Innate Immune Response			Adaptive Immune Response		
Cytokine	Crohn's disease	Ulcerative colitis	Cytokine	Crohn's disease	Ulcerative colitis
IL-1β	I	I	IL-5	N	I
IL-6	I	I	IL-13	N	I
IL-8	I	I	IL-17	I	N
IL-12	I	N	IL-21	I	N
IL-18	I	I	IFN-γ	I	N
IL-23	I	N	TL1α	I	?
IL-27	I	N			
TNF-α	I	I			
Light	I	I			
TL1α	I	?			

IL: Interleukin; TNF: Tumor necrosis factor; IFN: Interferon; I: Increase; N: Normal.

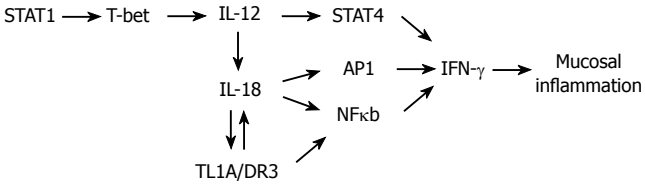

Figure 3 Th1 Polarization Pathway. Polarization of naïve T-cells towards Th1 cell subtype is initially induced by IL-12, a heterodimer of the p40 and p35 subunits. Activation of signal transducer and activator of transcription-1 (STAT1) and its stimulation of transcription factor T-bet, a TH1 "master switch" that upregulates and stabilizes the expression of IL-12. IL-12 can then amplify TH1 response by upregulating IL-18 on T-cells. IL-18 then stimulates NF-κB and AP1, and in synergy with STAT4 (activated by IL-12), transactivates IFN-γ expression.

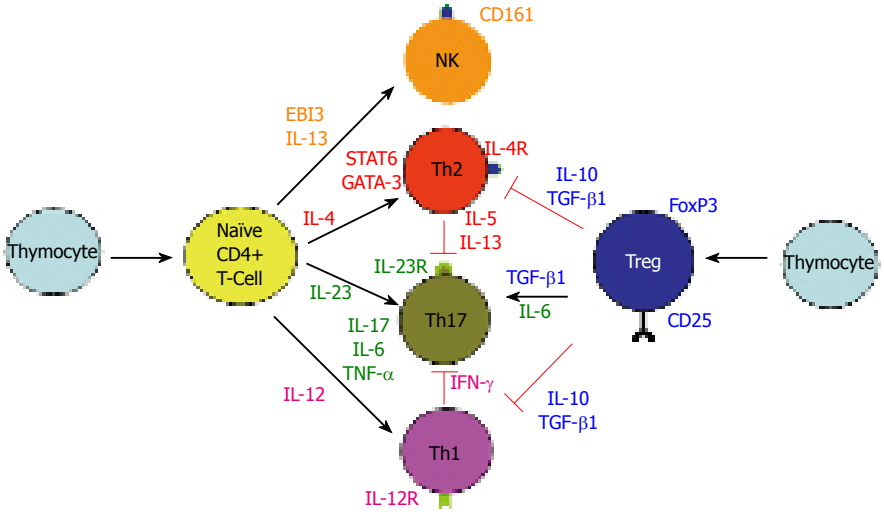

Figure 4 Differentiation of T-cell Subsets. Upon stimulation, naïve CD4+ T-cells differentiate into 3 main subsets, Th1, Th2, and Th17 cell. IL-12 induces the formation of IFN-γ producing Th1 cells. IL-23 promotes the development of an IL-17 producing CD4+ helper T cells. IL-4 induces STAT6 activation, promoting the expression of GATA-3, which feed forward to induce IL-4 expression and Th2 cell differentiation. An EBI3-associated cytokine was hypothesized to be necessary for activation of IL-13 producing NK T-cells. Tregs, an immune-modulating subset of CD4+ T-cells, can suppress the differentiation and function of Th1 and Th2 cells. Interestingly, in the presence of IL-6, Treg-derived TGF-β can induce the differentiation of Th17 cells.

the human TH1 mucosal response in CD. This genome-wide association revealed a highly significant association of SNP haplotypes of the TL1A gene with CD in a large cohort of Japanese patients as well as in two separate, smaller European cohorts[103]. These findings suggest that TNF mediated signaling is an important immune regulatory mechanism in mucosal inflammatory responses.

The IL-23/IL-17 immune axis

A subset of T cells producing IL-6 and IL-17, now known as Th17 cells, has emerged as an important mediator of the T-cell response in gut inflammation (Figure 4). IL-17 is a proinflammatory cytokine that enhances T-cell priming and stimulates fibroblasts, endothelial cells, macrophages, and epithelial cells to produce multiple pro-inflammatory mediators such as IL-1, IL-6, TNF, NOS2, metalloproteases, and chemokines[104]. IL-23 (composed of p19 and p40) promotes the development of an IL-17 producing CD4+ helper T cell subset through mechanisms that are distinct from the Th1 (STAT1, STAT4, and T-bet) and Th2 (STAT6) pathways[85,86,105]. Through this pathway, IL-23 regulates inflammatory processes in several mouse disease models[106-108]. In addition, bacterial colonization stimulates IL-23 expression by ileal dendritic cells[109] and the levels of both IL-23 and IL-17 are increased in Crohn's disease tissue[110,111].

CD is thought to be mediated by Th1 cells because of high levels of IL-12 and IFN-γ are detected and treatment with anti-IFN-γ mAb or anti-IL12 (p40) mAB suppresses disease development[112,113]. However, as IL-23 shares the p40 subunit with IL-12, the improved clinical disease may also be an effect of IL-23 inhibition. To determine the contribution of IL-23 in intestinal inflammation, mice with deficiency of IL-23 ($p19^{-/-}$) or IL-12 ($p35^{-/-}$) were used. A recent study showed that the development of colitis was suppressed by IL-23p19 deficiency but not IL-12p35 deficiency in $IL\text{-}10^{-/-}$ mice (model of T-cell mediated gut inflammation)[114]. Administration of IL-23 accelerated the onset of colitis in $Rag^{-/-}$ mice reconstituted with $IL\text{-}10^{-/-}$ CD4+ T-cells[114]. The suppression of inflammation in IL-23p19 deficient mice is not due to impaired Th1 (preserved IFN-γ expression) or Th2 (preserved IL-4 expression) but likely due to IL-17 pathway[114]. Notably, IL-23 (but not IL-12) augments IL-17 and -6 expressions by anti-CD3 mAB-stimulated memory CD4+ T-cells, distinguishing the ability of IL-12 to stimulate naïve CD4+ T-cells[114]. Antibodies that neutralize IL-6 and IL-17 ameliorated the severity of intestinal inflammation in $Rag^{-/-}$ mice reconstituted with $IL\text{-}10^{-/-}$ CD4+ T-cells[114]. These observations indicate that IL-23 promotes development and expansion of a pathogenic IL-6/IL-17 producing memory-activated T-cell population that can trigger the inflammatory cascade leading to intestinal inflammation.

The above findings do not exclude the role of exaggerated Th1 response in CD since IL-12/IFN-γ and IL-23/IL-17 may be parallel pathways involved in inflammatory response. Interestingly, IL-12/IFN-γ and IL-23/IL17 pathways are mutually exclusive, since IFN-γ suppress IL-17 and vice versa (Figure 4)[104]. It is of pathogenic importance to consider all of the major immune pathways that are responsible for the development of CD. For example, therapeutic targeting of only the newly discovered IL-23/IL-17 immune axis may actually exacerbate CD by accelerating the IL-2/IFN-γ Th1 pathway.

T-CELL RESPONSES IN ULCERATIVE COLITIS

In ulcerative colitis, it appears that the T-cell response is Th2 dominant (IL-4, IL-13) and mediated by specialized cells such as NK T-cells. By determining the cytokine profile of lamina propria mononuclear cells (LPMC) isolated from tissue recovered from colonic resection from UC and CD patients, it is found that LPMC from UC patients secreted high amounts of Th2 cytokines IL-13 and IL-5[115]. The IL-13 and -5 LPMC cells bear NK specific markers CD161 and recognizes CD1d, indicating that they are NK T-cells[115]. These NK T-cells are "nonclassical" because they do not express invariant NK T-cell receptors characteristic of most "classical" NK T-cells. They express noninvariant (diverse) TCRs that recognize antigens in association with CD1d, a MHC class I-like molecule present on the surface of dendritic cells

and on non-professional antigen presenting cells (APC) such as intestinal epithelial cells[116]. The "nonclassical" NK T-cells isolated from UC patients exhibited cytotoxicity towards an epithelial cell line (HT-29)[115]. This cell population possibly could be the cells causing epithelial cell cytotoxicity in UC described in 1960-1980s[117]. Together, these data show that ulcerative colitis is associated with an atypical Th-2 response mediated by a distinct subset of NK T-cells that produce IL-13 and are cytotoxic for epithelial cells. However, the extent to which this leads to the ultimate cascade of inflammation in ulcerative colitis remains to be determined.

Colonic epithelial cells express both CD1d and the Epstein-Barr virus-induced gene (EBI3), a protein related to IL-12p40. An EBI3-associated cytokine was hypothesized to be necessary for activation of IL-13 producing NK T-cells[118]. This is consistent with the fact that EBI3 is increased in mucosa from patients with UC as compared with CD or control tissues[119]. In addition, EBI3-deficient mice manifest poor Th2 responses and are resistant to the development of oxazolone colitis, a Th2 colitic murine model[120]. The EBI3-deficient mice have markedly reduced numbers of NK T-cells while the number of both naïve and mature CD4+ and CD8+ cells represerved[120]. These collective data indicate EBI3-associated cytokine (e.g. IL-27) may be necessary for the development of the Th2-cytokine-producing NK T-cells in both UC and oxazolone colitis.

CONCLUSION

Available evidence indicates that IBD is the result of dysregulated immunogenetic parameters that depends on impaired coordination between luminal microorganisms, gut epithelium, and the host immune system in genetically susceptible individual (Figure 1). These immunogenic parameters are complex and biologically divergent, which explains the heterogeneous clinical manifestations and lack of a universal therapeutic response to any single agent. The challenge for the future is to better understand the precise molecular mechanisms of disease immunopathogenesis. This can be achieved by identification of novel immunogenetic parameters, characterization of the heterogeneous parameters, and integrated multiparameter assessment of distinct aspects of the disease biology that are already known. These multi-disciplinary approaches are likely to stratify this complex disease into distinct subgroups that are more biologically homogenous with more defined pathogenic parameters and guidance for a more predictable response for individualized IBD therapy.

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